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Potential Beneficial Effects of Apelin-13 on Testicular Ischemia-Reperfusion Injury

Ayhan Tanyeli[1], Mustafa Can Guler[1] Ersen Eraslan[2], Saime Özbek Şebin[1], Burak Bircan[3], Engin Şebin[4] Fazile Nur Ekinci Akdemir[5*]

[1]Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye
[2]Department of Physiology, Faculty of Medicine, Bandırma OnYedi Eylül University, Bandırma, Türkiye
[3]Department of Medical Services, Vocational School of Health Services, Osmaniye Korkut Ata University, Osmaniye, Türkiye
[4]Department of Medical Biochemistry, Erzurum City Hospital, Erzurum, Türkiye
[5]Department of Nutrition and Dietetics, Faculty of Health Science, Ağrı İbrahim Çeçen University, Ağrı, Türkiye

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****Corresponding Author***
Fazile Nur Ekinci Akdemir
Department of Nutrition and Dietetics
Faculty of Health Science
Ağrı İbrahim Çeçen University
Ağrı, Türkiye
Phone: +90 5304333566
E-mail: fnekinciakdemir@gmail.com

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Authors' ORCIDs
Ayhan Tanyeli
https://orcid.org/0000-0002-0095-0917
Fazile Nur Ekinci Akdemir
http://orcid.org/0000-0001-9585-3169
Ersen Eraslan
http://orcid.org/0000-0003-2424-2269
Mustafa Can Güler
https://orcid.org/0000-0001-8588-1035
Saime Özbek Şebin
https://orcid.org/0000-0002-1738-4800
Burak Bircan
http://orcid.org/0000-0003-1141-069X
Engin Şebin
http://orcid.org/0000-0001-9150-8069

Abstract: Testicular ischemia-reperfusion (T I/R) injury leads to oxidative stress with excessive accumulation of reactive oxygen species in the tissue. This phenomenon has an essential place in the pathophysiology of testicular torsion injury. The presented study aimed to reveal the prophylactic beneficial effects of apelin 13 (APE-13) on T I/R damage. Twenty-four male Sprague Dawley rats were randomly divided into sham, I/R, 10µg/kg APE-13, and 100µg/kg APE-13 groups. I/R protocol and APE-13 application doses were applied in previous studies. At the end of the experiment, all rats were sacrificed, and their testicular tissues were quickly removed. It was stored under appropriate conditions until biochemical analysis was performed. In the biochemical analysis of the tissues, oxidative parameters and inflammatory cytokine levels increased, and antioxidant levels decreased in the testicular tissue due to I/R. On the other hand, these results changed significantly in the 10µg/kg and 100µg/kg APE-13 groups. Considering the presented data, the severity of T I/R-induced tissue damage was reduced when APE-13 was administered at doses of 10µg/kg and 100µg/kg. ©2024 NTMS.
Keywords: Apelin-13; Testis; Ischemia-Reperfusion.

1. Introduction

The clinical phenomenon defined as testicular torsion is the obstruction of blood flow to the testicles due to the twisting of the spermatic cord around its axis, insufficiency of metabolism, and deterioration of

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testicular function. Testicular torsion is a risk factor that can occur in all age groups but is more critical for newborns and young adults. Medical diagnosis should be made quickly, and surgical intervention should be performed as soon as possible to treat it [1, 2].

Testicular torsion and detorsion directly results in ischemia-reperfusion (I/R). In addition to causing infertility and testicular atrophy, I/R injuries can lead to fatal clinical events such as acute heart failure, acute myocardial infarction, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome and require urgent intervention [3-5]. Experimental and clinical studies have revealed that there is a significant relationship between male infertility and oxidative stress. This relationship is explained by the fact that free radicals produced intensively in the molecular processes of testicular torsion and I/R cause oxidative stress, causing damage to seminiferous tissues and resulting in sterility in men [4, 6]. Free radicals attack polyunsaturated fatty acids, cellular molecules, and DNA, causing intense lipid peroxidation. In this regard, the fact that spermatozoa have abundant polyunsaturated fatty acids causes them to be exposed to radical attacks and disrupt spermatogenesis [1, 7, 8]. Many researchers have experimentally tested drugs or agents with various pharmacological activities to alleviate or eliminate testicular I/R injury [1, 5, 8, 9]. Apelin, a peptide compound, is a pro-hormone with 77 amino acids and was first isolated from bovine stomach extract [10]. Various apelin isoforms, such as apelin-12, apelin-13, apelin-17, and apelin-36, are formed from preproapelin, a precursor protein containing 77 amino acids. [11]. Pyroglutamyl-apelin13 ([Pyr1] apelin-13), which is more resistant to enzymatic destruction, is formed from apelin-13 (APE-13) by posttranslational modification. Additionally, apelin and G-protein coupled apelin receptor APJ is expressed in various tissues containing the pancreas, brain, stomach, skeletal muscle, and heart and exerts various protective biological effects by inhibiting inflammation and attenuating apoptosis [12]. As a result of our extensive literature research, we could not find any study showing that APE-13 prophylactic application was tested in the T I/R model. Therefore, the presented study aimed to determine the possible beneficial effects of APE-13 application in alleviating T I/R damage.

2. Material and Methods

2.1. Experimental Procedure and Rats

The animals were obtained from Atatürk University Animal Experiments Research Center, and animal experiments were carried out at the same center. Additionally, ethical permissions for the study were obtained from Atatürk University Animal Experiments Local Ethics Committee (Date and number 30.03.2018/54). All experimental animals were kept under standard laboratory conditions (55% humidity, 25 degrees' temperature, 12/12 hours' dark/light cycle) and fed with standard pellet feed and tap water. The 24 Sprague Dawley male rats used in the study were weighed and randomly divided into four groups: sham, I/R, 10µg/kg APE-13, and 100µg/kg APE-13 groups. Since the sham group was the control group of this study, the I/R model or APE-13 doses weren't applied. Just to standardize the stress levels of animals in all groups, a median laparotomy incision of 1-2 cm in size was made and closed under anesthesia (ketamine/xylazine 60/10 mg/kg bw, intraperitoneally). in the sham group. Animals in the I/R group were anesthetized and fixed in a supine position, the incision area was cleaned with povidone-iodine solution, and a 1-2 cm incision was made. The spermatic cord was clamped by twisting at 720 degrees, thus initiating 2 hours of ischemia. At the end of the period, reperfusion was created by opening the clamp and re-blooding the testicles for 2 hours. The incision area was closed again. In the 10µg/kg APE-13 and 100µg/kg APE-13 groups, the experimental I/R model defined in the I/R group was created, and APE-13 was administered intraperitoneally to these groups at doses of 10 and 100 µg/kg 30 minutes before reperfusion. At the end of the experiments, the testicles were removed and stored under appropriate conditions until biochemical analysis. Notably, the experimental I/R model used in this study and the anesthesia, and the APE-13 doses used were chosen based on previous studies [2, 13, 14].

2.2. Biochemical Analysis

For biochemical analysis, myeloperoxidase (MPO) activity, malondialdehyde (MDA) level, and superoxide dismutase activity (SOD) in homogenized testicular tissues were studied according to the methods specified by Bradley et al., Sun et al., Ohkawa et al. [15-17]. These results were expressed as U/mg protein, nmol/g, and U/mg protein. Total antioxidant status (TAS), total oxidant status (TOS) values, Interleukin- 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) levels were measured using appropriate kits (Rel Assay Diagnosis and Elabscience, Wuhan, China). The oxidative stress index (OSI) calculation was expressed as the TOS/TAS ratio.

2.3. Statistical *Analysis*

SPSS 20 (SPSS Corporation, Chicago, IL, USA) statistical program was used for data analysis. The results were expressed as Mean±Standard Deviation (SD), and $p<0.05$ was considered statistically significant. One-way analysis of variance was used for statistical analysis, and the Tukey post hoc test was applied to determine the difference between groups.

3. Results

When the biochemical results announced in the presented study were evaluated for oxidative parameters, the OSI and TOS value, MDA level, and MPO activity increased dramatically in the I/R group compared to the sham group. It triggered an intense free radical production in the testicular tissue. In contrast, two doses of APE-13 were documented to reduce oxidative markers in the 10µg/kg APE-13 and

100µg/kg APE-13 groups compared to the I/R group. The results of the study's basic indicators of antioxidant defense showed that SOD activity and TAS levels were significantly reduced in the I/R group compared to the sham group. Also APE-13. It was observed that antioxidant defense in testicular tissue was supported depending on the application at doses of 10 and 100 µg/kg, and these parameters were increased in the 10 and 100 µg/kg APE-13 groups compared to the I/R group. In the evaluation of the levels of pro-inflammatory cytokines in this study, it was revealed that TNF-α and IL-1β levels increased critically in the I/R group compared to the sham group. Still, the cytokine levels decreased in the 10 and 100 µg/kg APE-13 groups (see Figure 1 and 2; Table 1).

Table 1: Comparison of TNF-α (pg/mg protein), and IL-1β (pg/mg protein) results of all groups.

	Mean	Standart Deviaton	Minimum	Maximum
TNF-α (pg/mg protein)				
Sham	23847.05	3744.80	18637.60	29834.20
T I/R	37750.80[a]	4572.82	28435.20	42156.60
10 µg/kg APE-13	2742802[b]	2824.40	22265.50	31178.60
100 µg/kg APE-13	24238.35[b]	3890.30	20367.60	30453.00
IL-1β (pg/mg protein)				
Sham	2652548	2356.75	23365.10	29895.80
T I/R	7280017[a]	4713.91	64356.10	78945.90
10 µg/kg APE-13	3770013[b]	6381.10	27785.50	47546.50
100 µg/kg APE-13	29473.01[b*]	2589.10	25567.40	32785.90

[a]p<0.001 comperative to Sham group, [b]p<0.001 comperative to T I/R. *p<0.001 comperative to 10 µg/kg APE-13 groups. Data are presented as Mean±SD.

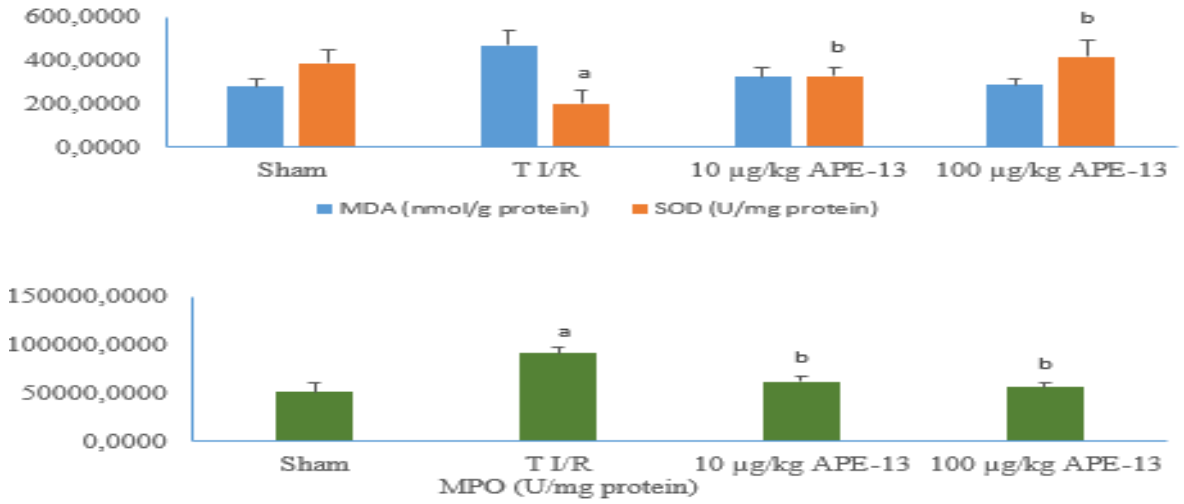

Figure 1: Comparison of MDA (nmol/g protein), SOD (U/mg protein) and MPO (U/mg protein) results of all groups. [a]p<0.001 comperative to Sham group, [b]p<0.001 comperative to T I/R. Data are presented as Mean ±SD.

4. Discussion

Current research reports that free radicals produced intensively during the I/R process directly cause testicular damage, apoptosis, and infertility [18]. The occurrence of consequences such as apoptosis, oxidative stress, and infertility varies in proportion to how long the blood flow of the testicular tissue is blocked and how fast the detorsion is made. The phenomenon of oxidative stress arises from the change in the balance between the amount of cellular oxidants and the cellular antioxidant defense system in favor of oxidants [19]. In this respect, it is critical to immediately restore the twisted testicles and apply drugs or agents that support antioxidant defense [2, 8, 9].

The primary marker of I/R-induced tissue damage is the MDA level. This marker describes lipid peroxidation, in which excessive amounts of free radicals produced in the tissue cause the peroxidation of cellular molecules [19]. As a result, oxidative stress and I/R damage are indicated by high MDA levels in the tissue. Antioxidants are defined as molecules that can prevent the oxidation of cellular molecules. Antioxidant compounds can scavenge free radicals, delay the lipid

peroxidation process, and protect the organism from radical damage. Moreover, they delay lipid peroxidation and the progression of many chronic diseases [19, 20]. Due to ischemia, various pro-inflammatory genes and transcription factors are upregulated in cells. In addition, the hypoxia-related decrease in ATP and glycogen content and the increase in testicular calcium ions (Ca^{2+}) are the critical points of testicular damage [21]. The increased cytokine production and adhesion molecule expression in the ischemic process by cells exposed to hypoxia/ischemia represents the main problem for direct reperfusion injury.

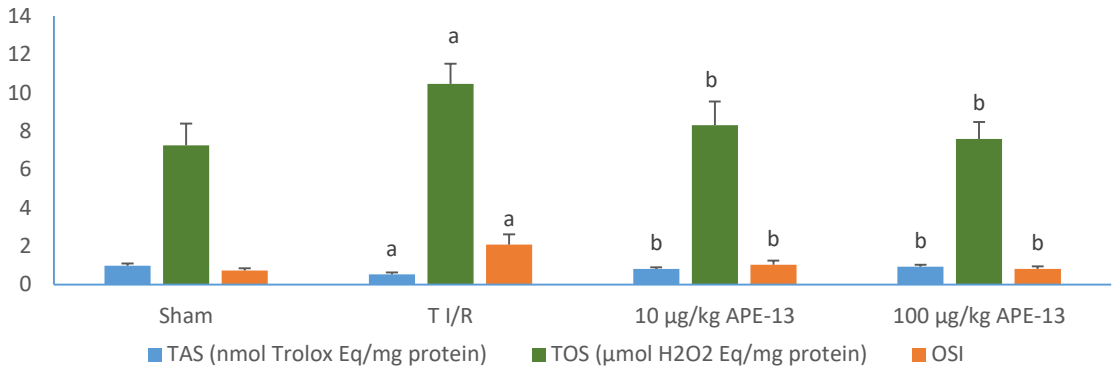

Figure 2: Comparison of TAS (nmol/Trolox Eq/mg protein), TOS (µmol H_2O_2 Eq/mg protein) and OSI levels of all groups. [a]p<0.001 comperative to Sham group, [b]p<0.001 comperative to T I/R goup. Data are presented as Mean±SD.

Moreover, the accumulation of neutrophils triggers an increase in MPO activities. Also, the accumulated neutrophils aggravate testicular damage by producing free radicals, TNF-α, and local inflammatory cytokines [22, 23]. Many studies on this subject have examined the effectiveness of different agents in alleviating T I/R-induced testicular tissue damage [7, 24]. In a few examples of studies on apelin-13, the experimental results of apelin-13 treatment provide valuable information. In one of the studies on I/R injury mitigation, Ape-13 inhibited excessive autophagy and apoptosis in cerebral ischemia/reperfusion injury [25]. Another study documented that Apelin-13 alleviates cerebral ischemia/reperfusion injury by regulating inflammation and the JAK2/STAT3 signaling pathway [14].

These studies also showed that apelin-13, TNFα, IL-1β, IL 6, and MDA levels were reduced, and the total antioxidant capacity level was increased in experimental cerebral ischemia models [14, 26]. In a study conducted on a different subject, it was reported that APE-13 suppresses the apoptotic pathway in cochlear damage caused by experimental noise exposure, reduces oxidative stress by increasing SOD activity, and thus improves cochlear damage [27]. In addition to these studies, APE-13 increased catalase activity in embryonic cardiomyocytes and decreased plasma lipid hydroperoxide levels, an essential oxidative stress finding [28]. These summarized studies showed that the severity of oxidative stress, inflammation, and apoptosis in the tissue decreased due to APE-13treatments. The findings presented in this study are compatible with the findings of various studies in the literature, and it has been proven in this study that APE-13 treatment managed to protect testicular tissue against T I/R damage significantly.

5. Conclusion

According to our literature research, this presented study is the first to reveal the protective effect of APE-13 against oxidative and inflammatory damage to testicular tissue in the T I/R rat model. The present study showed that APE-13 promoted antioxidant and anti-inflammatory status in testicular tissue in experimental animals exposed to T I/R and attenuated oxidative stress by limiting free radical production. In conclusion, APE-13 may be an effective therapeutic agent in preventing cell damage in T I/R-induced damaged testicular tissue, which may lead to improvement of the function of testicular tissue in rats. In this respect, APE-13 may serve as a therapeutic agent in the damage of testicular tissue in the future.

Limitations of the Study

Among the limitations of the study, financial inadequacies in advanced analyzes and measurement of a larger number of parameters can be mentioned.

Acknowledgement

None.

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

Conceived and designed the experiments; TA, MCG, EE, SÖŞ, BB and EŞ. Analyzed and interpreted the data; EE. Contributed reagents, materials, analysis tools or data; TA, MCG, SÖŞ, BB and EŞ. Wrote the paper; FNEA Study of biostatistics; FNEA and EE.

Ethical Approval

Ethical permissions for the study were obtained from Atatürk University Animal Experiments Local Ethics Committee (Date and number 30.03.2018/54).

Data sharing statement
All data relevant to the study are included in the article.
Consent to participate
None.
Informed Statement
None.

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